CONFIDENTIAL

Study code: 3000-4209
(Old code 3000-IIIF2-009)

Study title:

Hydroxymatairesinol. Modified Irwin screen test in the mouse.

CONFIDENTIAL

CONFIDENTIAL

RCC Study Number 780636

Hydroxymatairesinol:

Modified Irwin Screen Test
in the mouse

Final Report

Authors: Dr. K.M. Bray-French, M. Gisin

CONFIDENTIAL

RCC

TABLE OF CONTENTS

1 PREFACE 3
1.1 GENERAL 3
1.2 RESPONSIBILITIES 3
1.3 SCHEDULE 3
1.4 ARCHIVING 4
1.5 SIGNATURES 5
1.6 QUALITY ASSURANCE STATEMENT 6
1.7 STATEMENT OF COMPLIANCE 7
1.8 TEST GUIDELINE 8
2 PURPOSE 8
3 MATERIALS AND METHODS 8
3.1 TEST SYSTEM 8
3.2 ALLOCATION 9
3.3 HUSBANDRY 9
3.4 TEST ITEM 9
3.5 VEHICLE 10
3.6 DOSE FORMULATION 10
3.7 TREATMENT 10
3.8 PROCEDURE AND OBSERVATIONS 11
3.9 TERMINATION OF THE EXPERIMENT 15
3.10 DATA COMPILATION AND STATISTICAL ANALYSIS 15
4 SUMMARY OF RESULTS 16
5 CONCLUSION 16
6 SUMMARY TABLES 17
ATTACHMENT I:.- INDIVIDUAL VALUES 19
ATTACHMENT II: - ANALYTICAL STUDY REPORT 26
ATTACHMENT III:.- CERTIFICATE OF ANALYSIS 51
LAST PAGE OF REPORT 52

1 PREFACE

1.1 GENERAL

Title	Hydroxymatairesinol: Modified Irwin screen test in the mouse
Sponsor	Hormos Nutraceutical Ltd. Tykistökatu 6A, Biocity Turku, FIN-20520 Finland
Study Monitor	Dr. Helena Korte
Test Facilities	a, RCC Ltd Toxicology Division 4452 Itingen / Switzerland b, University of Turku, Institute of Biomedicine Dept. of Pharmacology and Clinical Pharmacology / BRST Kiinamyllynkatu 10 FIN-20520 TURKU Finland

1.2 RESPONSIBILITIES

Study Director	Dr. K.M. Bray-French (a)
Deputy Study Director	Dr. J. Laliberté (a)
Study Coordinator	M. Gisin (a)
Principal Investigator	P. Tapanainen (b). Analysis of dosing formulations performed under CRST Bioanalytics Study Code A165 and Sponsor Study Code 3000-IIIF2-009/A.
Head of RCC Quality Assurance	I. Wüthrich

1.3 SCHEDULE

Acclimatisation start	04 October, 2000
Experimental Starting Date	11 October, 2000
Experimental Completion Date	12 October, 2000
Final Report	02 October, 2001

1.4 ARCHIVING

RCC Ltd (4452 Itingen / Switzerland) will retain the study plan, raw data, a sample of test item and the final report of the present study for at least ten years. No data will be discarded without the Sponsor's consent.

1.5 SIGNATURES

Study Director: Dr. K.M. Bray-French

K M Bray-French

date: 02-OCT-2001

Deputy for Study Director: (for) Dr. J. Laliberté

date: 02-OCT-2001

Study Co-ordinator: M. Gisin

date: 02-Oct-2001

Management: S. J. Corney

S. Corney

date: 02-Oct-2001

1.6 QUALITY ASSURANCE UNIT

RCC LTD, TOXICOLOGY DIVISION, 4452 ITINGEN / SWITZERLAND

STATEMENT

RCC STUDY NUMBER:	780636
TEST ITEM:	Hydroxymatairesinol
STUDY DIRECTOR:	Dr. K.M. Bray-French
TITLE:	Hydroxymatairesinol: Modified Irwin screen test in the mouse

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Study procedures were periodically inspected. The study plan and this report were audited by the RCC Quality Assurance Unit. The dates are given below:

Dates and types of QAU inspections/audits		Dates of reports to the study director and to management
Dates	Types	
19-SEP-2000	Study Plan audit	19-SEP-2000
11-OCT-2000	Study based inspection (Test item / dose preparation / raw data / test system)	11-OCT-2000
07-DEC-2000	Report audit	07-DEC-2000
01-OCT-2001	Report audit	01-OCT-2001

This statement also confirms that this final report reflects the raw data.

Quality Assurance (for) P. Stolz

date: 02 October 2001

GOOD LABORATORY PRACTICE

1.7 STATEMENT OF COMPLIANCE

RCC STUDY NUMBER:	780636
TEST ITEM:	Hydroxymatairesinol
STUDY DIRECTOR:	Dr. K.M. Bray-French
TITLE:	Hydroxymatairesinol: Modified Irwin screen test in the mouse

This study was performed in compliance with:

Swiss Ordinance relating to Good Laboratory Practice, adopted February 2nd, 2000 [RS 813.016.5]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C (97)186/Final].

These procedures are consistent with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA), and Japan (MHW, MAFF and MITI).

There were no circumstances that may have affected the quality or integrity of the data.

Study Director: Dr. K.M. Bray-French

KM Bray-French

date: 02-OCT-2001

1.8 TEST GUIDELINE

Japanese Guidelines for the Nonclinical Studies of Drugs Manual 1995: Guidelines for General Pharmacology studies. Issued by the Ministry of Health and Welfare, Pharmaceutical Affairs Bureau, April 1995.

2 PURPOSE

The purpose of this study was to evaluate possible pharmacological effects of the test item in NMRI mice, when administered orally. To this end, the effect of the test item on the general behaviour of the mouse was investigated using a modified Irwin screen test for evaluation.

3 MATERIALS AND METHODS

3.1 TEST SYSTEM

Test system	NMRI mice, outbred, SPF quality
Rationale	Commonly used for studies of this type.
Source	RCC Ltd Biotechnology & Animal Breeding Division 4414 Füllinsdorf, Switzerland
Number of animals / group	3 males
Total number of animals	12 males
Age at delivery (ordered)	3 weeks
Age at treatment	4 weeks
Body weight range ordered	20 – 24 grams
Body weight range at treatment	26 – 30 grams (fasted)
Identification	Tail marking with indelible felt-tip pen
Number allocation	Selected by hand and allocated to home cage in a non-chronological fashion at delivery.
Acclimatization	7 days, after health examination.

3.2 ALLOCATION

Treatment	Group 1 Vehicle	Group 2 Hydroxy-matairesinol	Group 3 Hydroxy-matairesinol	Group 4 Hydroxy-matairesinol
Dose (mg/kg)	**0**	**1000**	**200**	**40**
Animal Number	1 – 3	4 – 6	7 - 9	10 - 12

*Because this test may be considered subjective in nature, the investigator performing the observations was not aware of the treatment administered to groups 2-4. The treatment given to these groups was randomised.

Doses are stated in terms of the test item as supplied.

3.3 HUSBANDRY

Room number	
- acclimatisation	128 B
- testing	128 B
Conditions	Standard Laboratory Conditions. Air-conditioned with 10-15 air changes per hour, and continuously monitored environment with target ranges for temperature 22 ± 3 °C and relative humidity 30-70%. 12 hours fluorescent light/12 hours dark (light period between 6.00 and 18.00), music during the light period.
Accommodation	Individually in Makrolon-type 2 cages with wire mesh tops and softwood bedding (Lignocel, Schill AG, 4132 Muttenz/ Switzerland).
Diet	Pelleted standard Provimi Kliba 3433 rat and mouse maintenance diet (Provimi Kliba AG, 4303 Kaiseraugst/ Switzerland) *ad libitum*. Batch No. 02/00: Results of representative analyses for contaminants are retained in the raw data.
Water	Community tap water from Itingen, *ad libitum*. Results of representative bacteriological, chemical and contaminants analyses are retained in the raw data.

3.4 TEST ITEM

(Information as provided by the sponsor)

Identity	Hydroxymatairesinol
Description	Solid, labile
Batch number	T011-B304P1
Purity	>95%

Molecular weight	374.39
Storage	In the refrigerator at 2 – 8°C
Expiry date	31 July, 2001
Safety precautions	Routine hygienic precautions were employed to assure personnel health and safety.

3.5 VEHICLE

Identity	Polyethylene glycol (PEG) 300
Source	Merck-Schuchardt, 85662 Hohenbrunn / Germany
Batch number	S95440 944
Expiry date	March 2004

3.6 DOSE FORMULATION

Doses are in terms of test item as supplied.

Prior to start of the study, a non-GLP pre-test was performed to verify the preparation of the dose formulations.

The test item was weighed into a container on a tared precision balance and the vehicle added. The mixture obtained was shortly sonicated until complete dissolution.
Further dilutions of this solution were made with vehicle.

Frequency of preparation	Once, just prior to treatment on the test day.
Storage of dose formulations	In the refrigerator at 2 – 8°C
Stability of dose formulations	Stable for at least 24 hours at 2 – 8°C (see Attachment II)
Analysis of dose formulations	Shortly after preparation and at 24 hours after preparation, aliquots of each dose formulation were frozen at -20°C and sent to the Principal Investigator Pasi Tapanainen or Kristo Hakala, University of Turku, Institute of Biomedicine, Dept. of Pharmacology and Clinical Pharmacology / BRST, Kiinamyllynkatu 10, 20520 Turku, Finland, for analysis of content and stability using HPLC-UV. The results of the analyses are reported in Attachment II to the report.

3.7 TREATMENT

Method / dose route	Oral (by gavage). Animals were fasted overnight with access to water *ad libitum*. Feed was re-presented at 4 hours after dosing.
Rationale	Intended clinical route of administration

Dose levels	Group 1: Vehicle Group 4: 40 mg/kg body weight Group 3: 200 mg/kg body weight Group 2: 1000 mg/kg body weight Each animal was weighed immediately before treatment and the dose was adjusted according to body weight. Doses are stated in term of test item as supplied.
Rationale for dose level selection	Requested by the Sponsor
Frequency of administration	Once to each animal.
Dose volume	10 ml/kg

3.8 PROCEDURE AND OBSERVATIONS

The following observations were recorded:

Detailed clinical signs	The signs described overleaf were recorded before and at 0.5, 1, 2, and 4 hours after treatment. Feed was re-presented at 4 hours after dosing.
Body weights	On test day prior to dosing

A. HOME CAGE

	Observation	Score (if any)		Remarks / explanation
a.	Increased locomotor activity	1 - 2	1 – 2 = increased activity	
b.	Abnormal behaviour: Type: Restlessness (NR) Writhing (W)	1	1 = present	NR - characterised by an appearance of uneasiness or discomfort with inability to remain long in a given position. W - characterised by an undulatory wave-like movement over the abdomen that involves a flattening of the abdominal wall accompanied by asymmetrical stretching and extending of the body and hind limbs.
c.	Alertness Increased or Decreased	1	1 = increased or decreased	Observed on opening the home cage.
d.	Startle response Increased or Decreased	1	1 = increased or decreased	A sudden body jerking movement of the animal in response to a noise stimulus.

B. IN THE ARENA

	Observation	Score (if any)		Remarks / explanation
a.	Pilo-erection	1 - 2	1 - 2 = increased pilo-erection	Erection of the hair characterised by ruffled fur, with (2) or without (1) a ball-like appearance.
b.	Tremor	1 - 2	1 – 2 = increased tremor	These are involuntary oscillatory movements which result from the alternate contraction of opposing muscle groups; especially noticeable on limbs and tail.
c.	Twitches	1	1 = present	These are brief, coarse involuntary muscle contractions which cause the animal to jerk or twitch its limbs and/or body.
d.	Respiration Type: A - abdominal G - gasping S - slower F - faster	1	1 = present	The nature of the respiratory changes will be noted.

	Observation	Score (if any)		Remarks / explanation
e.	Seizures Type: I - clonic II - tonic	1	1 = spasms present	
f.	Abnormal behaviour Type: A - apathy D - darting	1	1 = present	A - animal immobile with head lowered or resting on the bottom of the arena D - sudden directional running behaviour (often to the animals own peril)
g.	Abnormal body carriage Type: St - Straub tail Hu - hunched Hi - hind quarters raised Sh - shuffling (dragging hind limbs)	1 - 2	1 - 2 =increasing degree of abnormality	The nature of the abnormal body carriage and the intensity on a scale of 1 to 2 is noted.
h.	Abnormal gait Type: S - limbs spread further apart T - standing or walking on its toes W - waddling (rolling from side to side) L - walking low on limbs U - animal incapable of motor activity	1 - 2	1 - 2 =increasing degree of abnormality	The nature of the abnormal limb position and gait is noted; the intensity of the effect is also scored.
i.	Exploratory activity Increased or Decreased	1 - 2	1 – 2 = degree of activity	Exploration is indicated by the sniffing and examination of the arena.
j.	Sedation	1 - 2	1 – 2 = increased sedation	

C. IN THE HAND

	Observation	Score (if any)		Remarks / explanation
a.	Fearfulness	1	1 = present	
b.	Passivity	1 - 4	1 - 4 = increased passivity	Is scored on the basis of struggle behaviour exhibited when the animal is placed in various positions: Animal suspended vertically by the nape of the neck. Score 1 if struggle response shown, and 2 if not shown. Animal is rested across back of observers hand. Score 3 if no struggle response. Animal lifted and suspended by one fore- or hind-limb. Score 4 if no struggle response.
c.	Exophthalmos	1	1 = present	Abnormal protrusion or bulging of the eyeball.
d.	Pupil diameter Type: A = mydriasis B = miosis	1	1 = abnormal	Mydriasis = increase in pupil size Miosis = decrease in pupil size
e.	Response of pupil to light	1	1 = decreased pupil contraction response	
f.	Pinna reflex	1	1 = pinna reflex absent	The flicking or retraction of the ear in response to light touch stimulation of the external ear passage
g.	Corneal reflex	1	1 = corneal reflex absent	The blind or eye-closure response to light touch stimulation of the cornea
h.	Hypothermia (touch detection only)	1	1 = decreased temperature	
i.	Cutaneous blood flow Increased or Decreased	1	1 = increased or decreased blood flow	Scored in terms of the colour of the tail, plantar surface of the hind-limbs and the nose. Increased blood flow = vasodilation Decreased blood flow = vasoconstriction
j.	Cyanosis	1	1 = cyanosis	A bluish discoloration of the nose, limbs and tail

	Observation	Score (if any)		Remarks / explanation
k.	Ptosis	1 - 2	1 = ¼ - ½ closed 2 = ¾ closed to completely closed	This represents a closure or drooping of the upper eyelids.
l.	Lacrimation	1	1 = increased lacrimation	Occurrence of tear secretions from the eyes
m.	Salivation	1	1 = increased salivation	Increased saliva production indicated by moist fur around the mouth.
n.	Body tone Increased or Decreased	1	1 = increased or decreased body tone	Will be observed during the general handling of the animal.
o.	Pain response Increased or Decreased	1	1 = increased or decreased pain response	Response of the animal when hind-paw is pinched.
p.	Aggressiveness	1	1 = increased aggressiveness	Tendency of the animal to bite when mouth forcibly opened (e.g. with tweezers).
q.	Vocalisation	1 - 2	1 – 2 = increased vocalisation	The incidence of squeaking during animal handling is noted.
r.	Diarrhoea	1	1 = diarrhoea	
D. ON RETURN TO THE HOME CAGE				
a	Grooming Increased or Decreased	1	1 = increased (almost continuous) or decreased (almost no) grooming	The duration of the washing activity of the animals is observed after replacing the animal in the home cage.

3.9 TERMINATION OF THE EXPERIMENT

At the end of the test, all animals were killed by an intraperitoneal injection of sodium pentobarbitone followed by exsanguination.

3.10 DATA COMPILATION AND STATISTICAL ANALYSIS

For each animal, all behavioural findings observed were entered manually into a VAX computer system for summarisation.

The Fisher's exact test was used to determine statistically significant differences from control animals. Mean and standard deviation values of body weight measurements were also calculated for each group.

4 SUMMARY OF RESULTS

At 30 minutes, 1, 2 and 4 hours after a single oral administration of 40, 200 or 1000 mg/kg Hydroxymatairesinol, animals were assessed for behavioural changes. Observations included home cage assessments as well as the response to a new environment (arena) and handling.

Following administration of Hydroxymatairesinol at 40, 200 and 1000 mg/kg no overt behavioural changes were noted.

Before dosing and at 0.5 and 1 hour after dosing of 40 mg/kg Hydroxymatairesinol, 1 of 3 animals showed aggressive behaviour. This effect did not attain statistical significance, was also seen in one animal before dosing and was no longer evident at 2 hours after dosing. It is therefore not thought to be of pharmacological relavance.

Following 200 mg/kg, one of three animals showed an increase in alertness before dosing and at all time points up to 4 hours after dosing. As increased alertness was also seen in one of three vehicle treated animals, this effect is not thought to be related to treatment.

No behavioural effects were seen following oral administration of 1000mg/kg, for up to 4 hours after dosing.

Analysis of the dosing solutions determined that the test item in vehicle was stable for at least 24 hours at 2-8°C. Two of the samples appeared to be incorrectly labelled. However, the fact that the samples 4mg/ml (20-OCT-2000) and 100 mg/11-OCT-2000) were slightly yellowish, whereas all other samples were colourless, provides evidence that the samples 4mg/kg and 100 mg/kg from 12-OCT-2000 were inadvertently reversed (see Attachment II).

5 CONCLUSION

No overt effects on the central nervous system were seen following a single oral administration of Hydroxymatairesinol at 40, 200 or 1000 mg/kg in the male NMRI mouse.

6 SUMMARY TABLE

Page

Modified Irwin Screen: Summary 18

ODIFIED IRWIN SCREEN SUMMARY
ALES

	GROUP 1 VEHICLE	GROUP 2 1000 MG/KG	GROUP 3 200 MG/KG	GROUP 4 40 MG/KG
) FINDINGS				
BEFORE DOSING	1/3	3/3	2/3	2/3
0.5 HOUR AFTER DOSING	2/3	3/3	2/3	2/3
1 HOUR AFTER DOSING	2/3	3/3	2/3	2/3
2 HOURS AFTER DOSING	2/3	3/3	2/3	3/3
4 HOURS AFTER DOSING	3/3	3/3	2/3	3/3
.ERTNESS INCREASED				
BEFORE DOSING	1/3	0/3	1/3	0/3
0.5 HOUR AFTER DOSING	1/3	0/3	1/3	0/3
1 HOUR AFTER DOSING	1/3	0/3	1/3	0/3
2 HOURS AFTER DOSING	1/3	0/3	1/3	0/3
4 HOURS AFTER DOSING	0/3	0/3	1/3	0/3
;GRESSION				
BEFORE DOSING	1/3	0/3	0/3	1/3
0.5 HOUR AFTER DOSING	0/3	0/3	0/3	1/3
1 HOUR AFTER DOSING	0/3	0/3	0/3	1/3

'# : Fisher's exact test significant at level 5% (#) or 1% (##)